

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C. 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 September 1999 (06.09.99)	
International application No. PCT/US98/27629	Applicant's or agent's file reference GC500-2-PCT
International filing date (day/month/year) 23 December 1998 (23.12.98)	Priority date (day/month/year) 24 December 1997 (24.12.97)
Applicant SCHELLENBERGER, Volker et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
22 July 1999 (22.07.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

ANDERSON, Kirsten, A.
Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304-1013
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 28 January 2000 (28.01.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference GC500-2-PCT	
International application No. PCT/US98/27629	
International filing date (day/month/year) 23 December 1998 (23.12.98)	

1. The following indications appeared on record concerning:
- ☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address SCHELLENBERGER, Volker 1747 Sequoia Avenue Burlingame, CA 94010 United States of America	State of Nationality DE	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
- ☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address SCHELLENBERGER, Volker 914 Moreno Avenue Palo Alto, CA 94010 United States of America	State of Nationality DE	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

- ☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Athina Nickitas-Etienne
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

ANDERSON, Kirsten, A.
Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304-1013
ÉTATS-UNIS D'AMÉRIQUEDate of mailing (day/month/year)
28 January 2000 (28.01.00)Applicant's or agent's file reference
GC500-2-PCTInternational application No.
PCT/US98/27629

IMPORTANT NOTIFICATION

International filing date (day/month/year)
23 December 1998 (23.12.98)

1. The following indications appeared on record concerning:
- ☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

COLLIER, Katherine, D.
915 Wilmington Way
Redwood City, CA 94062
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
- ☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

COLLIER, Katherine, D.
915 Wilmington Way
Redwood City, CA 94070
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

- ☒ the receiving Office
☐ the International Searching Authority
☒ the International Preliminary Examining Authority

- ☐ the designated Offices concerned
☒ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

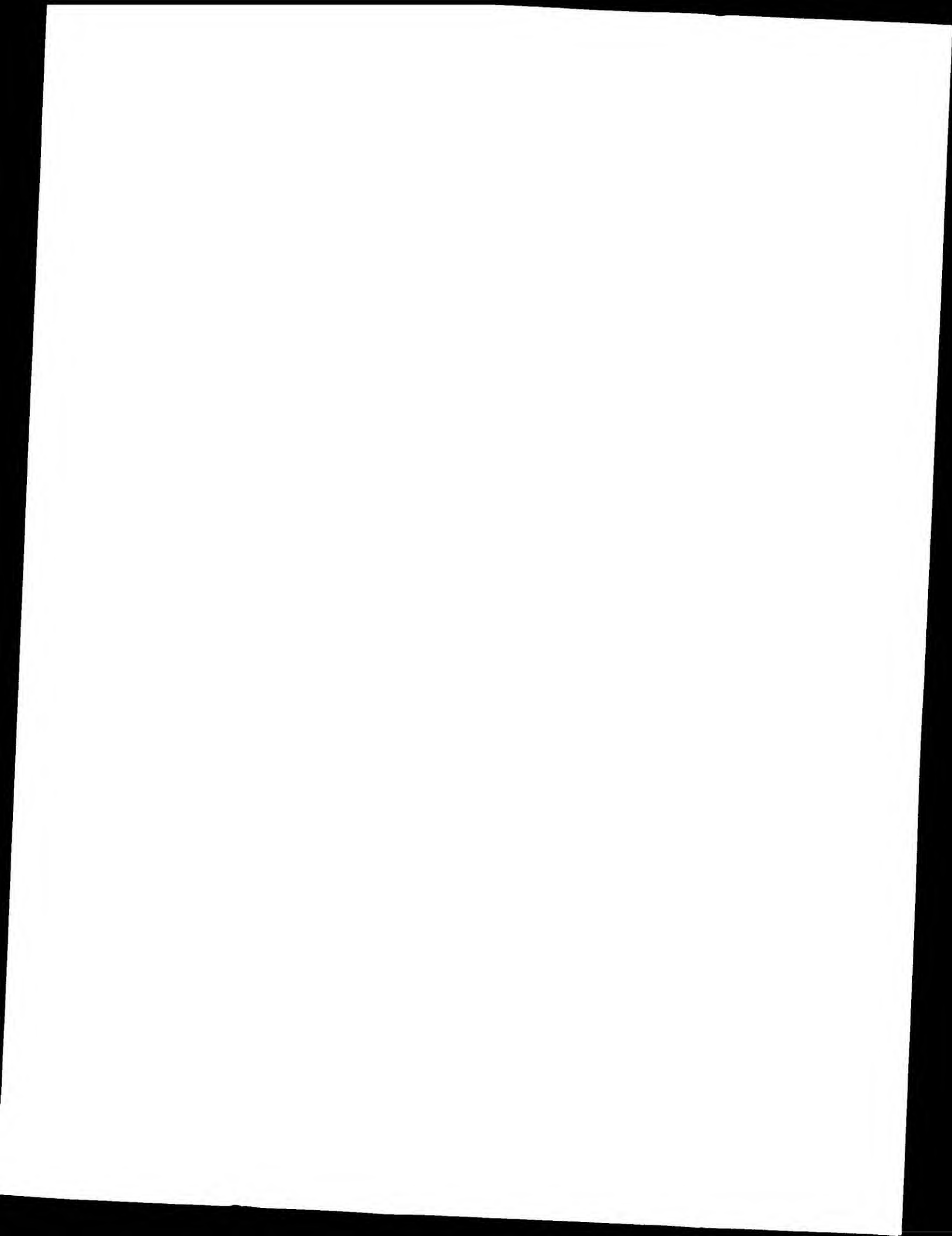
Facsimile No.: (41-22) 740.14.35

Authorized officer

Athina Nickitas-Etienne

Telephone No.: (41-22) 338.83.38

003076284



INTERNATIONAL SEARCH REPORT

National Application No

PCT/US 98/27629

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 11770 A (HANSEN PETER KAMP ;BAUDITZ PETER (DK); MIKKELSEN FRANK (DK); NOVON) 11 March 1999 (1999-03-11) page 3, line 15 - line 19 page 10, line 5 - line 11 example 3 claim 24 ---	1-7,12, 13,15-25
E	WO 99 11769 A (HANSEN PETER KAMP ;BAUDITZ PETER (DK); MIKKELSEN FRANK (DK); NOVON) 11 March 1999 (1999-03-11) claim 24 page 3, line 25-29 page 10, line 1 - line 7 example 3 ---	1-7,12, 13,15-25

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

19 August 1999

Date of mailing of the international search report

23. 11. 1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Routledge, B



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/27629

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 739 982 A (GENENCOR INT) 30 October 1996 (1996-10-30) example 4 ---	1-7,12, 13,15-25
X	EP 0 352 244 A (NOVONORDISK AS ;BEROL NOBEL NACKA AB (SE)) 24 January 1990 (1990-01-24) example 2 ---	1-7,12, 13,15-25
X	WO 97 41212 A (NOVONORDISK AS ;HIRAYAMA SATOSHI (JP); TAIRA RIKAKO (JP); BORCH KI) 6 November 1997 (1997-11-06) page 7, line 6 - line 14 page 9 page 17, line 9 -page 19, line 18 example 9 ---	1-7,12, 13,15-25
X	WO 97 23593 A (PROCTER & GAMBLE ;CULLEN KEVIN (GB)) 3 July 1997 (1997-07-03) page 3, paragraph 2 page 4, paragraph 1 -page 5, paragraph 3 page 11, paragraph 5 - paragraph 6 example 1 ---	1-7,12, 13,15-25
X	WO 97 07202 A (NOVONORDISK AS ;OKKELS JENS SIGURD (DK); SVENDSEN ALLAN (DK); BORC) 27 February 1997 (1997-02-27) page 158, line 27 -page 160, line 14 examples 5-8 ---	1-7,12, 13,15-25
X	WO 95 10615 A (GENENCOR INT) 20 April 1995 (1995-04-20) page 7, paragraph 1 page 8, paragraph 9 -page 9, paragraph 1 page 19, paragraph 3 page 21, paragraph 1 -page 22, paragraph 1 example 6 ---	1-7,12, 13,15-25
X	WO 93 05134 A (NOVONORDISK AS) 18 March 1993 (1993-03-18) example 3 ---	1-7,12, 13,15-25
X	US 5 612 306 A (O'BRIEN JEANNE A ET AL) 18 March 1997 (1997-03-18) column 5, line 15 - line 55 examples 1,2 -----	1-7,12, 13,15-25



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 27529

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-7, 12, 13, 15-25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see further information
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 12, 13, 15-25

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-7,12,13,15-25 (all in part)

The independent claims 1 and 15 (which would appear to be identical in scope) do not provide adequate specific special technical features of the invention to be able to make a clear distinction between the claimed invention and the prior art. The embodiments claimed in the dependent claims are concerned with known conventional features. Furthermore, the claims are of an excessively broad nature encompassing the use of any enzyme against any stain on any material.

The claims would appear to consist merely of making a comparison between enzymes using a conventional method of judging cleaning effectiveness to identify further suitable enzymes.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



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INTERNATIONAL SEARCH REPORT

I. Information on patent family members

International Application No

PCT/US 98/27629

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9911770	A	11-03-1999	AU 9061898 A	22-03-1999
WO 9911769	A	11-03-1999	AU 9061798 A	22-03-1999
EP 0739982	A	30-10-1996	AU 703309 B	25-03-1999
			AU 5569296 A	18-11-1996
			AU 710006 B	09-09-1999
			AU 5692796 A	18-11-1996
			BR 9608071 A	26-01-1999
			CA 2219245 A	31-10-1996
			CA 2222141 A	31-10-1996
			CN 1185179 A	17-06-1998
			CN 1185807 A	24-06-1998
			WO 9634092 A	31-10-1996
			EP 0828840 A	18-03-1998
			EP 0827534 A	11-03-1998
			JP 11503902 T	06-04-1999
			JP 11504062 T	06-04-1999
			NZ 306973 A	28-10-1999
			NZ 307548 A	28-01-1999
			WO 9634108 A	31-10-1996
			US 5856165 A	05-01-1999
			ZA 9603347 A	04-11-1996
			ZA 9603349 A	04-11-1996
EP 0352244	A	24-01-1990	JP 2041398 A	09-02-1990
			US 5156761 A	20-10-1992
WO 9741212	A	06-11-1997	AU 2382397 A	19-11-1997
			EP 0897423 A	24-02-1999
WO 9723593	A	03-07-1997	NONE	
WO 9707202	A	27-02-1997	AU 6655196 A	12-03-1997
			CN 1192780 A	09-09-1998
			EP 0851913 A	08-07-1998
			JP 11510699 T	21-09-1999
			AU 6414096 A	18-02-1997
			AU 6414196 A	18-02-1997
			CN 1193346 A	16-09-1998
			WO 9704078 A	06-02-1997
			WO 9704079 A	06-02-1997
			EP 0839186 A	06-05-1998
WO 9510615	A	20-04-1995	AU 700373 B	07-01-1999
			AU 8015794 A	04-05-1995
			BR 9407825 A	06-05-1997
			CA 2173973 A	20-04-1995
			CN 1133068 A	09-10-1996
			CZ 9601065 A	11-09-1996
			EP 0723590 A	31-07-1996
			FI 961631 A	15-04-1996
			JP 9504170 T	28-04-1997
			NO 961468 A	12-04-1996
			NZ 274998 A	24-04-1997
			PL 313942 A	05-08-1996
			ZA 9408086 A	07-06-1995



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INTERNATIONAL SEARCH REPORT

1. on patent family members

International Application No

PCT/US 98/27629

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9305134 A	18-03-1993	AT 179752 T	15-05-1999
		DE 69229119 D	10-06-1999
		EP 0603328 A	29-06-1994
		ES 2133328 T	16-09-1999
		FI 941147 A	10-03-1994
		JP 7502288 T	09-03-1995
		US 5468416 A	21-11-1995

US 5612306 A	18-03-1997	AU 687536 B	26-02-1998
		AU 2102595 A	09-10-1995
		CA 2161975 A	28-09-1995
		EP 0699227 A	06-03-1996
		JP 8511299 T	26-11-1996
		NZ 283076 A	24-02-1997
		WO 9525782 A	28-09-1995



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PATENT COOPERATION TREATY

PCT

REC'D 23 MAY 2000
WIPO

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GC500-2-PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US98/27629	International filing date (day/month/year) 23/12/1998	Priority date (day/month/year) 24/12/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/00		
Applicant GENENCOR INTERNATIONAL, INC. et al.		


- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 10 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

- This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 22/07/1999	Date of completion of this report 19.05.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Maucher, C Telephone No. +49 89 2399 7415





**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US98/27629

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-7 as originally filed

Claims, No.:

1-25 as originally filed

Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
☒ claims Nos. 1-7, 12-13, 15-25 (partly); 8-11, 14 (completely).

because:



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US98/27629

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- ☒ no international search report has been established for the said claims Nos. 1-7, 12-13, 15-25 (partly); 8-11, 14 (completely).

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-7, 12-13, 15-25 (partly).



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US98/27629

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	21-25
	No:	Claims	1-7, 12-13, 15-20
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7, 12-13, 15-25
Industrial applicability (IA)	Yes:	Claims	1-7, 12-13, 15-25
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US98/27629

Point III:

A partial search report has been issued for claims 1-7, 12-13 and 15-25. Therefore, examination of novelty and inventive step is restricted to the specific embodiments of claims 3-5, i.e. specific enzymes, materials and stains.

Point IV:

According to claims 8-11 and 14, a method for assaying for a preferred detergent composition comprising using a swatch of material having a stain fixed thereto is claimed. The IPEA does not consider the subject-matter of these claims to be linked by a single general inventive concept to the subject-matter of claims 1-7, 12-13 and 15-25. If the applicant wishes to proceed to the national phase before the EPO, an objection against claims 8-11 and 14 because of lack of unity will have to be awaited.

Point V:

Reference is made to the following documents:

- D1: EP-A-0 739 982
- D2: EP-A-0 352 244
- D3: WO-A-97 41212
- D4: WO-A-97 23593
- D5: WO-A-97 07202
- D6: WO-A-95 10615
- D7: WO-A-93 05134
- D8: US-A-5 612 306

D1 describes an antiredeposition test (page 3, lines 27-30), wherein a white cotton fabric was incubated with pigmented soil and other products in a detergent. A cellulase was added to the solution. Three different cellulases were tested: BCE103 (page 5, lines 54ff. to page 6, line 3), Kao Kac™ and Denimax Ultra™ MG (table 1, page 7) The incubation took place under agitating conditions (90rpm). As a control, the same

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US98/27629

incubation was carried out without cellulase addition. After the incubation, the fabric was rinsed with running cold water and then dried. The whiteness of the fabric was measured by remission using a colorimeter. The control value was subtracted from the sample value (page 6, last paragraph to page 7, 1. paragraph).

D2 discloses washing tests with different detergents containing Savinase (protease). Standard fabrics which were soiled with blood, milk and ink or cocoa, milk and sugar or polyester/cotton soiled with milk and ink were used in that test. Light reflectance was measured on washed fabric, soiled fabric and clean fabric. The % retention of detergency was calculated.

D3 refers to a detergent composition comprising a lipolytic enzyme (page 1, first lines). An Activity-in-Detergent-Assay (page 9) was performed with the lipolytic enzyme according to the invention of D3 and compared to a known enzyme Lipolase™ (page 33-34, example 9). Cotton swatches with olive oil are added to the detergent solution and stirred. Remaining detergent is removed by rinsing in tap water. Good washing performance is expressed by the hydrolysis of oil on textile swatches (abstract), i.e. the effect of lipolytic enzyme is measured by measuring the degree of hydrolysis (whole page 9).

D4 discloses a base laundry detergent composition comprising a protease. Swatches (white cotton sheets) were prepared with different stains like spinach, chocolate ice cream or EMPA blood. The swatches were added to the detergent composition which was stirred. After washing, the swatches were dried and assessed for removal of the stains by an expert panel using a four point Scheffé scale (pages 54-55).

In D5, the wash performance of lipolytic enzymes was tested using swatches stained with lard/sudan red. These swatches were incubated in a detergent composition comprising a lipolytic enzyme. After rinsing and drying of the swatches, the reflectance was measured at 460 nm. Afterwards the fatty matter was extracted from the swatches and the amount of fatty matter left on the swatches gravimetrically determined. Another determining method was thin layer chromatography and determination of the % removal of lard or the delta refractance (page 158, line 27 to page 160, line 14).

D6 discloses detergent solutions comprising subtilisin variants (proteases). These

solutions can also contain other enzymes like cellulases etc. (page 21, last paragraph). Wash performance tests were evaluated by measuring the removal of stain (blood/milk/carbon black) from cloth swatches using a detergent and adding an enzyme to the solution. After rinsing, drying and pressing, the reflectance from the swatches was measured on a Chroma Meter. The performance was reported as a percentage of the performance of a known protease and was calculated by dividing the amount of said protease by the amount of variant protease (page 33, 1. line to page 34, last full paragraph).

In D7 wash performance tests were accomplished on grass juice soiled cotton with detergent and added enzyme (page 6, line 23 to page 7, bottom). 3 different enzymes were tested (page 7, table 1). The % remission was determined at 460 nm. The proteolytic activity was determined with casein as substrate (page 6, line 15).

In D8 cotton swatches stained with grass stain were prepared. A stable enzyme-containing laundry (abstract) prespotting composition was applied to the swatch. Said composition contained at least one nonionic surfactant (column 3, line 20). The stain removal characteristics were rated on the 1 to 5 scale of the AATCC Stain release replica, with 1 being essentially no removal and 5 being complete removal (columns 7-8, example 1). Example 3 (column 9) shows the effect of different protease enzymes on stain removal.

1. Article 33(2) PCT

The following examination has been carried out on the available prior art resulting from an incomplete search due to the very broad scope of the claims.

- 1.1. The subject-matter of claims 1 and 15 is not novel (Article 33(2) PCT) in the light of any one document of D1-D8, since these documents disclose embodiments falling within the scope of these claims (see summaries above).
- 1.2. Claim 2 is not novel in the light of the available prior art, since D1 (table 1, page 7), D2 (page 5, line 49) and D8 (column 7, lines 54ff.) disclose the measurement of the degree of removal of the stain from the material.



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US98/27629

- 1.3. Claims 3, 13 and 16 are not novel, since the available prior art discloses the claimed method wherein the enzyme is a protease (D2, D4, D6- D8), a cellulase (D3) or a lipase (D5, D7) (see the above summaries of the documents).
- 1.4. Claims 4 and 17 are not novel, since the available prior art discloses a material which is a fabric (D1-D8; see the above summaries of the documents).
- 1.5. Claims 5 and 18 are not novel in view of the prior art, since the stain in the prior art is blood (D2, D4, D6), milk (D2, D6), ink (D2), grass (D7, D8), spinach (D4), gravy (D5), chocolate (D4), clay (D1, D6), pigment (D2), oil (D3) or a combination thereof (D2, D5) (see the above summaries of the documents).
- 1.6. The subject-matter of claims 6, 12 and 19 is not novel in the light of the prior art, since the enzyme is applied to a swatch in combination with a detergent in documents D1-D8 (see summaries above).
- 1.7. Claims 7 and 20 are not novel in the light of the prior art, since D1, D3 and D4 disclose the method including agitating the swatch and enzyme during incubation (see summaries above).
- 1.8. Claims 21-25 are novel (Article 33(2) PCT) in view of the closest prior art, since the claimed features are not disclosed in any one of the documents D1- D8 (see summaries above).
2. Article 33(3) PCT
 - 2.1. The enzymes amylase and laccase (claim 3), materials such as plastic, glass and ceramic (claim 4) and stains such as egg, cheese and combinations of stains (claim 5) appear not to have been searched. If this subject-matter were novel and if the claims had been restricted to said subject-matter, the technical problem to be solved could only be seen in providing a method with alternative enzymes, materials or stains, leading to a method having the same effects as the methods already known in the prior art (see D1-D8). Thus, the claims would not have been considered as being inventive (Article 33(3) PCT).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US98/27629

- 2.2. The subject-matter of claims 21-25 is not inventive (Article 33(3) PCT), since these claims appear to contain only minor variations of a known method (see 1.1.-1.7.).

Point VI:

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO99/11769	11.3.1999	19.8.1998	29.8.1997
WO99/11770	11.3.1999	19.8.1998	29.8.1997

The above mentioned documents may be relevant in the regional phase.

Point VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the documents D1-D8 have not been identified in the description and the relevant background art disclosed therein has not been briefly discussed.
2. The following description passages are not clear with respect to the swatch containing a stain. It cannot be revealed from said passages whether the swatch initially contains a stain and a second stain is added during the procedure or whether the swatch does not contain any stain at the beginning and is stained later on with only one stain: page 1, lines 23-28, page 2, lines 18-21, page 3, lines 10-15 (see also VIII, 2.).
3. The unit "gallon" employed for instance on page 7, line 8, is not recognized in international practice, contrary to the requirements of Rule 10.1(d) PCT.
4. References to patent application numbers (e.g. page 5, line 8) have not been

replaced by the corresponding publication numbers.

Point VIII:

1. Claim 1 is unclear (Article 6 PCT) in scope, because the terms "material", "enzyme" and "stain" are too broad in scope. In fact, said claim encompasses any possible material, stain and enzyme.
2. Claims 1 and 15 are not clear (Article 6 PCT), because it appears from examples on page 6, lines 25-30 and page 7, lines 2-16 of the description that the swatch comprises only one stain. Therefore, said stain, which is bound to the swatch in step b) may not be already present in the same swatch of step a).
3. Claim 1 is furthermore unclear (Article 6 PCT), since it does not disclose how a preferred enzyme is assayed. Some steps of the method seem to be missing.
4. It is not clear to what the feature "preferred enzyme" corresponds in claim 1.
5. It is clear from the description on page 4, lines 13-17, that the following feature is essential to the definition of the invention:

"agitating the swatch and enzyme during incubation" (dependent claims 7 and 20).

Since independent claims 1 and 15 do not contain this feature, they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

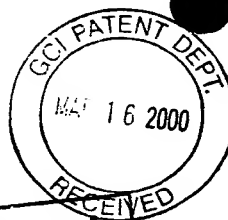
TENT COOPERATION TRE...

From the: *SF/kw*
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

FARIS, Susan et al.
GENENCOR INTERNATIONAL, INC.
925 Page Mill Road
Palo Alto, California 94304-1013
ETATS-UNIS D'AMERIQUE

by fax and post



cont copy

Resp Due ~~4/6/00~~

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Previously Vouched
WRITTEN OPINION

(PCT Rule 66)

Fax : 650-845-6504

Date of mailing
(day/month/year)

06.03.2000

Applicant's or agent's file reference

GC500-2-PCT

REPLY DUE

within 1 month(s)
from the above date of mailing

International application No.

PCT/US98/27629 ✓

International filing date (day/month/year)

23/12/1998

Priority date (day/month/year)

24/12/1997

International Patent Classification (IPC) or both national classification and IPC

C12Q1/00

Applicant

GENENCOR INTERNATIONAL, INC. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain document cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 24/04/2000.

Name and mailing address of the international preliminary examining authority:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner

Maucher, C

Formalities officer (incl. extension of time limits)

Danti, B

Telephone No. +49 89 2399 8161



WRITTEN OPINION

International application No. PCT/US98/27629

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-7 as originally filed

Claims, No.:

1-25 as originally filed

Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 1-7, 12-13, 15-25 (partly); 8-11, 14 (completely),

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

WRITTEN OPINION

International application No. PCT/US98/27629

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1-7, 12-13, 15-25 (partly); 8-11, 14 (completely).

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-7, 12-13, 15-25 (partly).

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7, 12-13, 15-20
Inventive step (IS)	Claims	1-7, 12-13, 15-25
Industrial applicability (IA)	Claims	1-7, 12-13, 15-25



2. Citations and explanations**see separate sheet****VI. Certain documents cited****1. Certain published documents (Rule 70.10)**

and / or

2. Non-written disclosures (Rule 70.9)**see separate sheet****VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

see separate sheet**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Point V:

Reference is made to the following documents:

- D1: EP-A-0 739 982
- D2: EP-A-0 352 244
- D3: WO-A-97 41212
- D4: WO-A-97 23593
- D5: WO-A-97 07202
- D6: WO-A-95 10615
- D7: WO-A-93 05134
- D8: US-A-5 612 306

D1 describes an antiredeposition test (page 3, lines 27-30), wherein a white cotton fabric was incubated with pigmented soil and other products in a detergent. A cellulase was added to the solution. Three different cellulases were tested: BCE103 (page 5, lines 54ff. to page 6, line 3), Kao Kac™ and Denimax Ultra™ MG (table 1, page 7) The incubation took place under agitating conditions (90rpm). As a control, the same incubation was carried out without cellulase addition. After the incubation, the fabric was rinsed with running cold water and then dried. The whiteness of the fabric was measured by remission using a colorimeter. The control value was subtracted from the sample value (page 6, last paragraph to page 7, 1. paragraph).

D2 discloses washing tests with different detergents containing Savinase (protease). Standard fabrics which were soiled with blood, milk and ink or cocoa, milk and sugar or polyester/cotton soiled with milk and ink were used in that test. Light reflectance was measured on washed fabric, soiled fabric and clean fabric. The % retention of detergency was calculated.

D3 refers to a detergent composition comprising a lipolytic enzyme (page 1, first lines). An Activity-in-Detergent-Assay (page 9) was performed with the lipolytic enzyme according to the invention of D3 and compared to a known enzyme Lipolase™ (page 33-34, example 9). Cotton swatches with olive oil are added to the detergent solution and stirred. Remaining detergent is removed by rinsing in tap water. Good washing performance is expressed by the hydrolysis of oil on textile swatches (abstract), i.e. the

effect of lipolytic enzyme is measured by measuring the degree of hydrolysis (whole page 9).

D4 discloses a base laundry detergent composition comprising a protease. Swatches (white cotton sheets) were prepared with different stains like spinach, chocolate ice cream or EMPA blood. The swatches were added to the detergent composition which was stirred. After washing, the swatches were dried and assessed for removal of the stains by an expert panel using a four point Scheffé scale (pages 54-55).

In D5, the wash performance of lipolytic enzymes was tested using swatches stained with lard/sudan red. These swatches were incubated in a detergent composition comprising a lipolytic enzyme. After rinsing and drying of the swatches, the reflectance was measured at 460 nm. Afterwards the fatty matter was extracted from the swatches and the amount of fatty matter left on the swatches gravimetrically determined. Another determining method was thin layer chromatography and determination of the % removal of lard or the delta refractance (page 158, line 27 to page 160, line 14).

D6 discloses detergent solutions comprising subtilisin variants (proteases). These solutions can also contain other enzymes like cellulases etc. (page 21, last paragraph). Wash performance tests were evaluated by measuring the removal of stain (blood/milk/carbon black) from cloth swatches using a detergent and adding an enzyme to the solution. After rinsing, drying and pressing, the reflectance from the swatches was measured on a Chroma Meter. The performance was reported as a percentage of the performance of a known protease and was calculated by dividing the amount of said protease by the amount of variant protease (page 33, 1. line to page 34, last full paragraph).

In D7 wash performance tests were accomplished on grass juice soiled cotton with detergent and added enzyme (page 6, line 23 to page 7, bottom). 3 different enzymes were tested (page 7, table 1). The % remission was determined at 460 nm. The proteolytic activity was determined with casein as substrate (page 6, line 15).

In D8 cotton swatches stained with grass stain were prepared. A stable enzyme-containing laundry (abstract) prespotting composition was applied to the swatch. Said composition contained at least one nonionic surfactant (column 3, line 20). The stain



removal characteristics were rated on the 1 to 5 scale of the AATCC Stain release replica, with 1 being essentially no removal and 5 being complete removal (columns 7-8, example 1). Example 3 (column 9) shows the effect of different protease enzymes on stain removal.

1. Article 33(2) PCT

The following examination has been carried out on the available prior art resulting from an incomplete search due to the very broad scope of the claims.

- 1.1. The subject-matter of claims 1 and 15 does not appear to be novel (Article 33(2) PCT) in the light of any one document of D1-D8, since these documents disclose embodiments falling within the scope of these claims (see summaries above).
- 1.2. Claim 2 is does not appear to be novel in the light of the available prior art, since D1 (table 1, page 7), D2 (page 5, line 49) and D8 (column 7, lines 54ff.) disclose the measurement of the degree of removal of the stain from the material.
- 1.3. Claims 3, 13 and 16 are not considered as being novel, since the available prior art discloses the claimed method wherein the enzyme is a protease (D2, D4, D6-D8), a cellulase (D3) or a lipase (D5, D7) (see the above summaries of the documents).
- 1.4. Claims 4 and 17 are not considered to be novel, since the available prior art discloses a material which is a fabric (D1-D8; see the above summaries of the documents).
- 1.5. Claims 5 and 18 do not appear to be novel in view of the prior art, since the stain in the prior art is blood (D2, D4, D6), milk (D2, D6), ink (D2), grass (D7, D8), spinach (D4), gravy (D5), chocolate (D4), clay (D1, D6), pigment (D2), oil (D3) or a combination thereof (D2, D5) (see the above summaries of the documents).
- 1.6. The subject-matter of claims 6, 12 and 19 is not considered novel in the light of the prior art, since the enzyme is applied to a swatch in combination with a

detergent in documents D1-D8 (see summaries above).

- 1.7. Claims 7 and 20 do not appear to be novel in the light of the prior art, since D1, D3 and D4 disclose the method including agitating the swatch and enzyme during incubation (see summaries above).
- 1.8. Claims 21-25 are considered novel (Article 33(2) PCT) in view of the closest prior art, since the claimed features are not disclosed in any one of the documents D1-D8 (see summaries above).

2. Article 33(3) PCT

- 2.1. The enzymes amylase and laccase (claim 3), materials such as plastic, glass and ceramic (claim 4) and stains such as egg, cheese and combinations of stains (claim 5) appear not to have been searched. If this subject-matter were novel and if the claims were restricted to said subject-matter, the technical problem to be solved could only be seen in providing a method with alternative enzymes, materials or stains, leading to a method having the same effects as the methods already known in the prior art (see D1-D8). Thus, the claims would not be considered as being inventive (Article 33(3) PCT).
- 2.2. The subject-matter of claims 21-25 is not considered to be inventive (Article 33(3) PCT), since these claims appear to contain only minor variations of a known method (see 1.1.-1.7.).



**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/US98/27629

Point VI:

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO99/11769	11.3.1999	19.8.1998	29.8.1997
WO99/11770	11.3.1999	19.8.1998	29.8.1997

The above mentioned documents may be relevant in the regional phase.

Point VII:

1. To meet the requirements of Rule 5.1(a)(ii) PCT, the documents D1-D8 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.
2. The definition of the term "swatch" (e.g. page 3, line 10) is not clear (see VIII, 1.).
3. The unit "gallon" employed for instance on page 7, line 8, is not recognized in international practice, contrary to the requirements of Rule 10.1(d) PCT.
4. References to patent application numbers (e.g. page 5, line 8) have to be replaced by the corresponding publication numbers.
5. It is not at present apparent which part of the application could serve as a basis for a new, allowable claim. Should the applicant nevertheless regard some particular matter as patentable an independent claim including such matter should be filed taking account of Rule 6.3(b) PCT.

The applicant should also indicate in the letter of reply the difference of the subject-matter of the new claim vis-à-vis the state of the art and the significance thereof.



6. Should the applicant however wish to proceed, he should take care during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Article 34(2)(b) PCT).
7. In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

These indications should be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Point VIII:

1. Claim 1 does not meet the requirements of Article 6 PCT, since the term "swatch", being defined as comprising a material and a stain, is unclear. In claims 1 and 15, for instance, a stain is fixed to a material (b)) already comprising a stain (a)). It however appears from the description that the swatch is treated with one stain only (page 1, lines 29-30). It appears furthermore from the prior art, that a swatch is synonymous with a sample of a material and not with a material and a stain. The applicant is invited to clarify this point.
2. Claim 1 is furthermore unclear (Article 6 PCT), since it does not disclose how a preferred enzyme is assayed. Some steps of the method seem to be missing.
3. It is not clear to what the feature "preferred enzyme" corresponds in claim 1.
4. It is clear from the description on page 4, lines 13-17, that the following feature is essential to the definition of the invention:

"agitating the swatch and enzyme during incubation" (dependent claims 7 and 20).

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/US98/27629

Since independent claims 1 and 15 do not contain this feature, they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

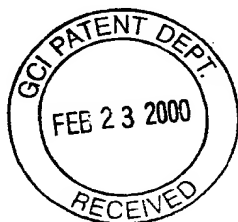
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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/00		A3	(11) International Publication Number: WO 99/34011
			(43) International Publication Date: 8 July 1999 (08.07.99)
(21) International Application Number: PCT/US98/27629		(81) Designated States: AL, AM, AT, AT (Utility model), AU (Petty patent), AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 23 December 1998 (23.12.98)			
(30) Priority Data: 60/068,796 24 December 1997 (24.12.97) US			
(71) Applicant (for all designated States except US): GENENCOR INTERNATIONAL, INC. [US/US]; 4 Cambridge Place, 1870 South Winton Road, Rochester, NY 14618 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): SCHELLENBERGER, Volker [DE/US]; 1747 Sequoia Avenue, Burlingame, CA 94010 (US). NAKI, Donald, P. [US/US]; 4815 - 25th Street, San Francisco, CA 94118 (US). COLLIER, Katherine, D. [US/US]; 915 Wilmington Way, Redwood City, CA 94062 (US). KELLIS, James, T., Jr. [US/US]; 111 Tan Oak Drive, Portola Valley, CA 94028 (US). NADHERNY, Joanne [US/US]; 681 Arguello No. 6, San Francisco, CA 94118 (US).		Published With international search report.	
(74) Agent: ANDERSON, Kirsten, A.; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013 (US).		(88) Date of publication of the international search report: 10 February 2000 (10.02.00)	
(54) Title: METHOD OF ASSAYING FOR A PREFERRED ENZYME AND/OR DETERGENT			
(57) Abstract An improved method for assaying the wash performance of new enzymes and/or new detergent formulations by comparing performance of enzyme cleaning effectiveness on washed soiled swatch cloths.			



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/US98/27629</p> <p>(22) International Filing Date: 23 December 1998 (23.12.98)</p> <p>(30) Priority Data: 60/068,796 24 December 1997 (24.12.97) US</p> <p>(71) Applicant (for all designated States except US): GENENCOR INTERNATIONAL, INC. [US/US]; 4 Cambridge Place, 1870 South Winton Road, Rochester, NY 14618 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): SCHELLENBERGER, Volker [DE/US]; 1747 Sequoia Avenue, Burlingame, CA 94010 (US). NAKI, Donald, P. [US/US]; 4815 - 25th Street, San Francisco, CA 94118 (US). COLLIER, Katherine, D. [US/US]; 915 Wilmington Way, Redwood City, CA 94062 (US). KELLIS, James, T., Jr. [US/US]; 111 Tan Oak Drive, Portola Valley, CA 94028 (US). NADHERNY, Joanne [US/US]; 681 Arguello No. 6, San Francisco, CA 94118 (US).</p> <p>(74) Agent: ANDERSON, Kirsten, A.; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013 (US).</p>		<p>(81) Designated States: AL, AM, AT, AT (Utility model), AU (Petty patent), AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>												
<p>(54) Title: AN IMPROVED METHOD OF ASSAYING FOR A PREFERRED ENZYME AND/OR PREFERRED DETERGENT COMPOSITION</p>														
<table border="1"><caption>Data points from the scatter plot</caption><thead><tr><th>Tergotometer (Reflectance L Value)</th><th>Microswatch (Abs. 620 nm)</th></tr></thead><tbody><tr><td>45.5</td><td>0.065</td></tr><tr><td>48.8</td><td>0.078</td></tr><tr><td>49.2</td><td>0.075</td></tr><tr><td>51.8</td><td>0.085</td></tr><tr><td>53.0</td><td>0.095</td></tr></tbody></table>			Tergotometer (Reflectance L Value)	Microswatch (Abs. 620 nm)	45.5	0.065	48.8	0.078	49.2	0.075	51.8	0.085	53.0	0.095
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<p>(57) Abstract</p> <p>An improved method for assaying the wash performance of new enzymes and/or new detergent formulations is described.</p>														

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AN IMPROVED METHOD OF ASSAYING FOR A PREFERRED ENZYME AND/OR
PREFERRED DETERGENT COMPOSITION

Background of the Invention

5 Enzymes are a necessary part of many of the detergent compositions that are currently on the market and the inclusion of enzymes in detergent compositions will undoubtedly increase in the future. One of the most important challenges facing a detergent manufacturer today is the identification of new and improved enzymes and detergent compositions. New enzymes can and commonly do include variants of known enzymes.

10 Several factors can affect the determination of the "improvement" of a new enzyme over an precursor enzyme, i.e., the enzyme itself, the wash conditions, and the detergent composition that the enzyme is to be mixed with. For example, an enzyme that performs well in one detergent composition may not perform as well in another. Similarly, an enzyme and/or detergent composition may perform well under one set of wash conditions, i.e., Japanese, but not another, i.e., North American. However, identifying a new and improved enzyme or detergent composition can be a time consuming task. For example, in the wake of improved technology that can allow a researcher to produce large numbers of variants in a very short time, it has become critical for the researcher to be able to assay those variants rapidly, efficiently and effectively.

Summary of the Invention

15 The present invention provides a method of assaying for a preferred enzyme including providing a swatch that includes a piece of material and a stain. The stain is then fixed to the material and a smaller swatch can be removed from the swatch. Alternatively, the smaller swatch can be removed from the larger swatch and then the stain can be fixed. Next, an enzyme is applied to the swatch or smaller swatch and they are incubated together.

30 The method can further include measuring the degree of removal of the stain from the material. The method can also include agitating the smaller swatch and enzyme during incubation. The material can be, for example, cotton, polyester or mixtures of natural and synthetic fibers. The stain can include blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof. The enzyme can be applied to the swatch or smaller swatch in combination with a detergent ingredient.

35 The present invention also provides a method of assaying for a preferred detergent composition including providing a swatch that includes a piece of material and a stain. The

stain is then fixed to the material and a smaller swatch can be removed from the swatch. Alternatively, the smaller swatch can be removed from the larger swatch and then the stain can be fixed. Next, a detergent composition is applied to the swatch or smaller swatch and they are incubated together.

5 The method can further include measuring the degree of removal of the stain from the material. The method can also include agitating the swatch or smaller swatch and detergent composition during incubation. The material can be, for example, cotton, polyester or mixtures of natural and synthetic fibers, cellulose and derivatives of cellulose. The stain can include blood, milk, ink, grass, spinach, wine, tea, gravy, chocolate egg,
10 cheese, clay, pigment, oil, and combinations thereof. The detergent composition can be applied to the swatch or smaller swatch in combination with an enzyme.

Brief Description of the Drawing

Figure 1 shows the correlation between the results of testing six protease variants
15 in a tergotometer test according to the method of the present invention.

Detailed Description of the Invention

One aspect of the present invention is directed to a method of assaying for a preferred enzyme that includes providing a swatch of material - a piece of material and a
20 stain - then fixing the stain to the material, optionally removing a smaller swatch from the swatch, applying the enzyme to the swatch or smaller swatch and incubating them.

A further aspect of the invention is directed to a method of assaying for a preferred detergent composition that includes providing a swatch of material that includes a piece of material and a stain, then fixing the stain to the material, optionally removing a smaller
25 swatch from the swatch, applying the detergent composition to the swatch or smaller swatch and incubating them.

Another aspect of the invention is directed to a method of assaying the release of a stain from a blood/milk/ink (BMI)-stained swatch including measuring the absorbance or fluorescence of, for example, the ink, labeled blood or labeled milk in the supernatant after
30 the swatch has been incubated with an enzyme or detergent composition.

In addition, an aspect of the invention includes a method of agitating the microtiter plate to a sufficient degree to assure complete and efficient incubation of the enzyme with the smaller swatch. The method includes applying a plate sealer to the top of the microtiter plate and then clamping another lid on top of the plate sealer.

35 Any enzyme or combination of enzymes may be used in the present invention. Preferred enzymes include those enzymes capable of hydrolyzing substrates, e.g. stains.

These enzymes are known as hydrolases which include, but are not limited to, proteases (bacterial, fungal, acid, neutral or alkaline), amylases (alpha or beta), lipases, cellulases and mixtures thereof. Particularly preferred enzymes are subtilisins and cellulases. Most preferred are subtilisins such as described in U.S. Patent 4,760,025, EP Patent 130 756 B1 and EP Patent Application WO 91/06637, which are incorporated herein by reference, and cellulases such as Multifect L250™ and Puradax™, commercially available from Genencor International. Other enzymes that can be used in the present invention include oxidases such as laccases, transferases, dehydratases, reductases, hemicellulases and isomerases.

10 A "swatch" is a piece of material such as a fabric that has a stain applied thereto. The material can be, for example, fabrics made of cotton, polyester or mixtures of natural and synthetic fibers. The swatch can further be paper such as filter paper or nitrocellulose or a piece of a hard material such as ceramic or glass. The stain can be blood, milk, ink, grass, tea, wine, spinach, gravy, chocolate egg, cheese, clay, pigment, oil, or mixtures of
15 these compounds.

A "smaller swatch" is a piece of the swatch that has been cut or otherwise removed from the swatch of material either before or after fixing the stain to the swatch and can, for example, fit into the well of a 24, 48 or 96 well microtiter plate. The "smaller swatch" can also be made by applying a stain to a small piece of material. Preferably, the smaller
20 swatch is a piece of fabric with a stain 5/8" in diameter, more preferably, the smaller swatch is 0.25" in diameter.

When, for example, untreated BMI swatches are washed in detergent without bleach, a large portion of the ink is released even without the help of a protease. Adding a protease leads to a small increase in ink release which can be hard to quantify over the
25 large background. The present invention provides a treatment protocol which allows one to control the degree of fixation of a stain. As a result, it is possible to produce swatches which, for example, release varying amounts of ink when washed in the absence of protease. The use of fixed swatches leads to a dramatic improvement of the signal-to-noise ratio in the wash assays. Furthermore, by varying the degree of fixation one can
30 generate stains which give optimum results under the various cleaning conditions.

Swatches having stains of known "strength" on various types of material are commercially available (EMPA, St. Gallen, Switzerland; wfk - Testgewebe GmbH, Krefeld Germany; or Center for Test Materials, Vlaardingen, The Netherlands) and/or can be made by the practitioner (Morris and Prato, Textile Research Journal 52(4):280-286 (1982)).
35 Preferred swatches are a blood/milk/ink (BMI) stain on a cotton-containing fabric, a spinach

stain on a cotton-containing fabric, or grass on a cotton-containing fabric, and chocolate/milk/soot on a cotton-containing fabric.

A stain can be fixed to a material in a number of ways. For example, the swatch can be incubated with a cross-linking agent to fix the stain. The degree of fixing can be affected by, for example, increasing or decreasing the incubation time, varying the temperature at which the incubation takes place, and/or varying the concentration of the chemical. Suitable cross-linking agents for use in the present invention include hydrogen peroxide, bleaching agents, glutaraldehyde, and carbodiimides.

In a preferred embodiment of the invention, a BMI stain can be fixed to cotton with 0.0003 - 0.3% hydrogen peroxide. Other combinations include grass or spinach fixed with 0.001-1% glutaraldehyde, gelatin and Coomassie stain fixed with 0.001-1% glutaraldehyde, or chocolate, milk and soot fixed with 0.001-1% glutaraldehyde.

An important aspect of the present invention is that the swatch and enzyme and/or detergent formulation must be well agitated during incubation. We have observed that the wash performance data is dependent on the orientation of the swatches in the wells (horizontal versus vertical), particularly in the 96-well plate. This would indicate that mixing was insufficient during the incubation period. Although there are a number of ways to ensure sufficient agitation during incubation, a plate holder in which the microtiter plate is sandwiched between two plates of aluminum can be constructed. This can be as simple as placing, for example, an adhesive plate sealer over the wells then clamping the two aluminum plates to the 96-well plate with any type of appropriate, commercially available clamps. It can then be mounted in a commercial incubator shaker. Setting the shaker to about 400 rpm results in very efficient mixing while leakage or cross-contamination is efficiently prevented by the holder.

Trinitrobenzenesulfonic acid (TNBS) can be used to quantify the concentration of amino groups in the wash liquor. This can serve as a measure of the amount of protein that was removed from the swatch (see Cayot and Tainturier, Anal. Biochem. 249:184-0200 (1997)). However, if a detergent or an enzyme sample leads to the formation of unusually small peptide fragments (for example, from the presence of peptidases in the sample) then one will obtain a larger TNBS signal, i.e., more "noise".

The present invention provides another and better way to measure wash performance of blood/milk/ink that is based on ink release. Proteolysis of protein on the swatches leads to the release of ink particles which can be quantified by measuring the absorbance of the wash liquor. The absorbance can be measured at any wavelength between 350 and 800 nm. In a preferred embodiment, the wavelength is measured at 410 nm. or 620 nm. The wash liquor can also be examined to determine the wash performance

on stains containing grass, spinach, gelatin or Coomassie stain. Preferred wavelengths for these stains include and 670nm for spinach or grass and 620nm for gelatin or Coomassie. For example, an aliquot of the wash liquor (typically 100 - 150ul from a 96-well microplate, for example) is removed and placed in a cuvette or multiwell microplate. This is
5 then placed in a spectrophotometer and the absorbance is read at an appropriate wavelength

The performance of samples of variant proteases (produced, for example, according to the disclosure of U.S. Patent Application Ser. No. 322,678) by the method of the present invention using TNBS and ink release detection can be compared. Several of
10 these samples show inflated wash performance when TNBS detection is used (probably due to peptidase contamination) whereas all samples result in indistinguishable signals when the absorbance of the wash liquor was measured.

The present invention can also be used to determine a preferred enzyme and/or detergent composition for dish washing, for example, using a blood/milk/ink stain on a
15 suitable substrate such as cloth, plastic or ceramic.

In a preferred embodiment of the invention, a BMI stain is fixed to cotton by applying 0.3% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 25°C or by applying 0.03% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 60°C. Smaller swatches of approximately 0.25" are cut from the BMI/cotton swatch and placed in
20 the wells of a 96 well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at 620nm is
25 measured.

In a further preferred embodiment of the invention, a spinach or grass stain is fixed to cotton by applying 0.01% glutaraldehyde to the spinach/cotton swatch or grass/cotton swatch for 30 minutes at 25°C. Smaller swatches of approximately 0.25" are cut from the swatch and placed in the wells of a 96 well microtiter plate. Into each well, a known
30 mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at 670nm is measured.

35 In another preferred embodiment of the invention, a chocolate/milk/soot stain is fixed to cotton by applying 0.01% glutaraldehyde to the chocolate/milk/soot/cotton swatch

30 minutes at 25°C. Smaller swatches of approximately 0.25" are cut from the swatch and placed in the wells of a 96 well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at an appropriate wavelength is measured.

Examples

Example I

A. Description of Tergotometer Protocol

A Tergotometer instrument manufactured by United States Testing Company was used. The machine consists of four or six 1.5 liter beakers and agitator spindles which are inserted into the beakers and rotated in a back-and forth manner at a controlled speed, typically 100 RPM, to mimic the type of agitation that occurs in commercial washing machines. The beakers are immersed in a temperature controlled water bath.

Each beaker was filled with one liter of deionized water to which a controlled amount of calcium and magnesium were added to mimic water hardness conditions found in the geography under study. Water hardness for North American conditions was set to 3 - 6 grains per gallon. The water bath was set to 20°C and the temperature of the water in the beakers was allowed to reach equilibrium at the testing temperature.

1 gram of Tide laundry detergent lacking bleach and enzyme (Procter & Gamble, Cincinnati, Ohio) was added to each beaker and allowed to mix for 1 minute while the spindles were rotating at 100 RPM. The enzyme was added to a final concentration of 0.1 micrograms per milliliter and allowed to mix for 1 minute. Blood-Milk-Ink soiled swatches 3" x 4 1/2" obtained from EMPA and modified by exposure to 3.0 % hydrogen peroxide for 30 minutes at 20°C and dried, were used. Six soiled swatches were added to each beaker and allowed to incubate for 20 minutes. After the incubation period the swatches were promptly removed from the beakers and rinsed thoroughly with water. The swatches were then placed flat on a clean lab bench to dry. When the swatches were dry, the reflectance of each swatch was measured at 3 different spots on each swatch, using a reflectance spectrophotometer with a small (typically 1/4") diameter aperture, capable of reporting results in the standard LAB scale. For BMI, it is sufficient to report only the L value, which correlates with the darkness of the stain. The L values obtained from the swatches in each pot were averaged to obtain the final reported result.

B. Description of 24-well Assay Protocol:

Blood-Milk-Ink swatches were obtained from EMPA and were exposed to 0.03 % hydrogen peroxide for 30 minutes at 60°C, then dried. Circles of ¼" diameter were cut from the dried swatches and placed one per well in a 24 well microplate. 1 gram per liter Tide laundry detergent without bleach and enzyme was prepared in deionized water, and a concentrated stock of calcium and magnesium was added to result in a final water hardness value of 6 grains per gallon. The detergent was allowed to mix for 15 minutes and was then filtered through a 0.2 micron cellulose acetate filter. Enzyme was added to the filtered detergent from a concentrated stock solution to result in a final concentration of 1.25 micrograms per milliliter. The enzyme/detergent solution was then added to the appropriate wells of the microplate. The microplate was then sealed to prevent leakage and placed in a holder on an incubated shaker set to 20°C and 400 RPM and allowed to shake for one hour. The plate was then removed from the incubator/shaker and an aliquot of 20 microliters was removed from each well, and the absorbance at 620 nm wavelength was read for each aliquot and reported.

C. Six protease variants were tested according to A and B above. The results are shown in Table 1. The correlation of the data is plotted in Figure 1. The R² value is 0.9652.

Table 1
Tergotometer Microswatch

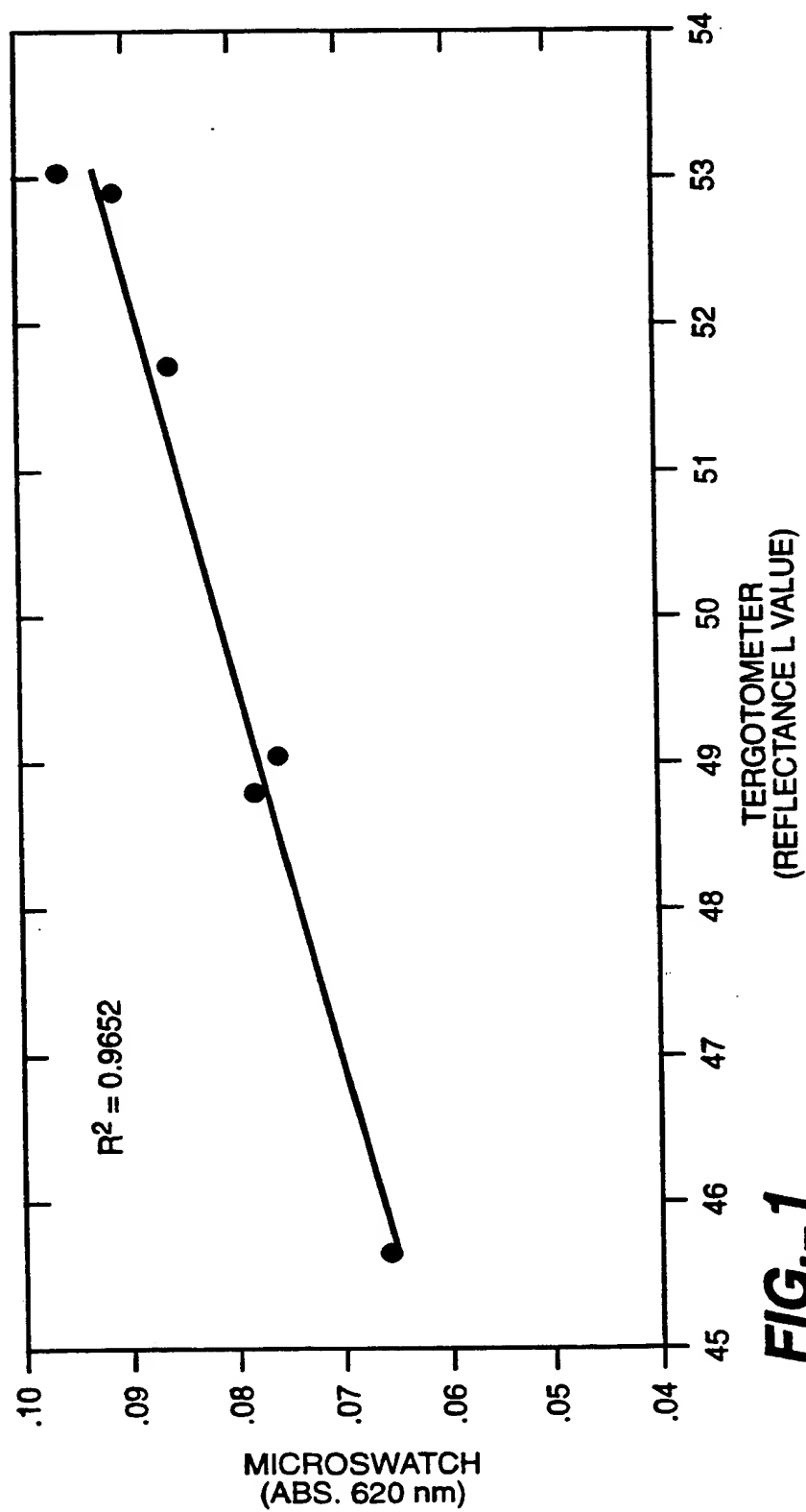
	L Value	Absorbance 620nm
A	45.62	0.066
B	48.815	0.078
C	51.755	0.086
D	49.06	0.076
E	52.915	0.091
F	53.065	0.096

Claims

1. A method of assaying for a preferred enzyme comprising:
 - a) providing a swatch of material comprising a piece of material and a stain;
 - b) fixing the stain to the material;
 - c) applying an enzyme to the swatch; and
 - d) incubating the swatch and enzyme.
2. The method of claim 1, further comprising measuring the degree of removal of the stain from the material.
3. The method of claim 1, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.
4. The method of claim 1, wherein the material is selected from the group consisting of a fabric, plastic, glass or ceramic.
5. The method of claim 1, wherein the stain is selected from the group consisting of blood, milk, ink, grass, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.
6. The method of claim 1, wherein the enzyme is applied to the swatch in combination with a detergent ingredient.
7. The method of claim 1, further comprising agitating the swatch and enzyme during incubation.
8. A method of assaying for a preferred detergent composition comprising:
 - a) providing a swatch of material comprising a piece of material and a stain;
 - b) fixing the stain to the material;
 - c) applying a detergent composition to the swatch; and
 - d) incubating the swatch and detergent composition.
9. The method of claim 8, further comprising measuring the degree of removal of the stain from the material.

10. The method of claim 8, wherein the material is selected from the group consisting of a fabric, plastic, glass, or ceramic.
11. The method of claim 8, wherein the stain is selected from the group consisting of blood, milk, ink, grass, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.
12. The method of claim 8, wherein the detergent composition is applied to the swatch in combination with an enzyme.
13. The method of claim 12, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.
14. The method of claim 8, further comprising agitating the swatch and detergent composition during incubation.
15. A method of determining the catalytic efficiency of an enzyme comprising:
 - a) providing a swatch of material comprising a piece of material and a stain;
 - b) applying the enzyme to the swatch;
 - c) incubating the swatch and enzyme;
 - d) removing the swatch or supernatant; and
 - e) measuring a constituent of the stain.
16. The method of claim 15, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.
17. The method of claim 15, wherein the material is selected from the group consisting of a fabric, plastic or ceramic.
18. The method of claim 15, wherein the stain is selected from the group consisting of blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.

19. The method of claim 15, wherein the enzyme is applied to the swatch in combination with a detergent ingredient.
20. The method of claim 15, further comprising agitating the swatch and enzyme during incubation.
21. The method of claim 15, wherein the constituent is ink from a BMI stain.
22. The method of claim 15, wherein the constituent is labeled blood from a BMI stain.
23. The method of claim 15, wherein the constituent is in the supernatant.
24. The method of claim 15, wherein the constituent is measured by absorbance of the constituent.
25. The method of claim 15, wherein the constituent is measured by the fluorescence of the constituent.

**FIG. 1**

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